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The Patent Office

Request for grant of a Patent

Form 1/77

Patents Act 1977

❶ Title of invention

1 Please give the title of the invention: Isolation of Nucleic Acid

❷ Applicant's details

❑ **First or only applicant**

2a If you are applying as a corporate body please give:

Corporate name: Dynal AS

Country *(and State of incorporation, if appropriate)*: Norway

2b If you are applying as an individual or one of a partnership please give in full:

Surname

Forenames

2c **In all cases**, please give the following details:

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N-0212 Oslo 2

UK postcode *(if applicable)*

Country: Norway

ADP number *(if known)*: 4309191001

❹ Reference number

4 Agent's or applicant's reference number *(if applicable)* 27.61695

❺ Claiming an earlier application date

5 Are you claiming that this application be treated as having been filed on the date of filing of an earlier application?

Please mark correct box

Yes ☐ No ☒ ➡ ***go to 6***

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❑ number of earlier application or patent number

❑ filing date

(day month year)

❑ and the Section of the Patents Act 1977 under which you are claiming.

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15(4) (Divisional) ☐ 8(3) ☐ 12(6) ☐ 37(4) ☐

❻ Declaration of priority

❻ If you are declaring priority from a PCT Application please enter 'PCT' as the country and enter the country code (for example, GB) as part of the application number.

Please give the date in all number format, for example, 31/05/90 for 31 May 1990.

6 If you are declaring priority from previous application(s), please give:

Country of filing	Priority application number *(if known)*	Filing date *(day, month, year)*
None		

61695.580

Isolation of Nucleic Acid

The present invention relates to the isolation of nucleic acid, and especially to the isolation of DNA from cells.

The isolation of DNA or RNA is an important step in many biochemical and diagnostic procedures. For example, the separation of nucleic acids from the complex mixtures in which they are often found is frequently necessary before other studies and procedures eg. cloning, sequencing, amplification, hybridisation etc. can be undertaken; the presence of large amounts of cellular or other contaminating material eg. proteins, in such complex mixtures often impedes many of the reactions and techniques used in molecular biology. Thus, methods for the isolation of nucleic acids from complex mixtures such as cells, tissues etc. are demanded, not only from the preparative point of view, but also in the many methods in use today which rely on the identification of DNA or RNA eg. diagnosis of microbial infections, forensic science, tissue and blood typing, detection of genetic variations etc.

A range of methods are known for the isolation of nucleic acids, but generally speaking, these rely on a complex series of extraction and washing steps and are time consuming and laborious to perform. Moreover, the use of materials such as alcohols and other organic solvents, chaotropes and proteinases is often involved, which is disadvantageous since such materials tend to interfere with many enzymic reactions and other downstream processing applications.

Thus, classical methods for the isolation of nucleic acids from complex starting materials such as blood or blood products or tissues involves lysis of the biological material by a detergent in the presence of

acid may be isolated from a sample in a form suitable for amplification or other downstream processes, by a simple and easy to perform procedure which involves treating the sample with detergent and allowing the nucleic acid to bind to a solid support, whereupon the nucleic acid may be readily separated from the sample, eg. by removal of the support. The binding of the nucleic acid is independent of its sequence.

In one aspect, the present invention thus provides a method of isolating nucleic acid from a sample, said method comprising contacting said sample with a detergent and a solid support, whereby soluble nucleic acid in said sample is bound to the support, and separating said support with bound nucleic acid from the sample.

The nucleic acid may be DNA, RNA or any naturally occurring or synthetic modification thereof, and combinations thereof. Preferably however the nucleic acid will be DNA, which may be genomic, or, cDNA, and single or double stranded or in any other form.

The samples may be any material containing nucleic acid, including for example foods and allied products, clinical and environmental samples. However, the sample will generally be a biological sample, which may contain any viral or cellular material, including all prokaryotic or eukaryotic cells, viruses, bacteriophages, mycoplasmas, protoplasts and organelles. Such biological material may thus comprise all types of mammalian and non-mammalian animal cells, plant cells, bacteria, protozoa etc. Representative samples thus include whole blood and blood-derived products such as plasma, serum and buffy coat, urine, faeces, cerebrospinal fluid or any other body fluids, tissues, cell cultures, cell suspensions etc.

The sample may also include relatively pure starting materials such as a PCR product, or semi-pure preparations obtained by other nucleic acid recovery

The detergent may be any detergent, and a vast range are known and described in the literature. Thus, the detergent may be ionic, including anionic and cationic, non-ionic or zwitterionic. The term "ionic detergent" as used herein includes any detergent which is partly or wholly in ionic form when dissolved in water. Anionic detergents have been shown to work particularly well and are preferred. Suitable anionic detergents include for example sodium dodecyl sulphate (SDS) or other alkali metal alkylsulphate salts or similar detergents, sarkosyl, or combinations thereof.

Conveniently, the detergent may be used in a concentration of 0.5 to 30% (w/v), preferably 1 to 15%, more preferably 1 to 10%. For anionic detergents concentrations of 1 to 5% have been shown to work well.

The detergent may be supplied in simple aqueous solution, or more preferably in a buffer. Any suitable buffer may be used, including for example Tris, Bicine, Tricine, and phosphate buffers. Conveniently, a source of monovalent cations, eg. a salt, may be included to enhance nucleic acid capture, although this is not necessary. Suitable salts include chloride salts, e.g. sodium chloride, lithium chloride etc. at concentrations of 0.1 to 1M, eg. 250 to 500 mM. As mentioned above, other components such as enzymes, may also be included.

Other optional components in the detergent composition include chelating agents eg. EDTA, EGTA and other polyamino carboxylic acids conveniently at concentrations of 1 to 50 mM etc., reducing agents such as dithiotreitol (DTT) or β-mercaptoethanol, at concentrations of for example 1 to 10 mM.

Preferred detergent compositions may for example comprise:

100 mM Tris-HCl pH 7.5
10 mM EDTA
2% SDS

beads. The size of the beads is not critical, but they may for example be of the order of diameter of at least 1 and preferably at least 2 μm, and have a maximum diameter of preferably not more than 10 and more preferably not more than 6 μm. For example, beads of diameter 2.8 μm and 4.5 μm have been shown to work well.

Monodisperse particles, that is those which are substantially uniform in size (eg. size having a diameter standard deviation of less than 5%) have the advantage that they provide very uniform reproducibility of reaction. Monodisperse polymer particles produced by the technique described in US-A-4336173 are especially suitable.

Non-magnetic polymer beads suitable for use in the method of the invention are available from Dyno Particles AS (Lillestrøm, Norway) as well as from Qiagen, Pharmacia and Serotec.

However, to aid manipulation and separation, magnetic beads are preferred. The term "magnetic" as used herein means that the support is capable of having a magnetic moment imparted to it when placed in a magnetic field, and thus is displaceable under the action of that field. In other words, a support comprising magnetic particles may readily be removed by magnetic aggregation, which provides a quick, simple and efficient way of separating the particles following the nucleic acid binding step, and is a far less rigorous method than traditional techniques such as centrifugation which generate shear forces which may degrade nucleic acids.

Thus, using the method of the invention, the magnetic particles with nucleic acid attached may be removed onto a suitable surface by application of a magnetic field eg. using a permanent magnet. It is usually sufficient to apply a magnet to the side of the vessel containing the sample mixture to aggregate the particles to the wall of the vessel and to pour away the

acids. For example, DNA binding proteins may be used, or viral proteins binding to viral nucleic acid. The attachment of such proteins to the solid support may be achieved using techniques well known in the art.

Although not necessary, it may be convenient to introduce one or more washing steps to the isolation method of the invention, for example following separation of the support from the sample. In the case of magnetic beads, this may conveniently be done before releasing the DNA from the beads. Any conventional washing buffers or other media may be used. Generally speaking, low to moderate ionic strength buffers are preferred eg. 10 mM Tris-HCl at pH 8.0/10mM NaCl. Other standard washing media, eg. containing alcohols, may also be used, if desired.

Following the separation step, and any optional washing steps which may be desired, the support carrying the nucleic acid may be transferred eg. resuspended or immersed into any suitable medium eg. water or low ionic strength buffer. Depending on the support and the nature of any subsequent processing desired, it may or may not be desirable to release the nucleic acid from the support.

In the case of a particulate solid support such as magnetic or non-magnetic beads, this may in many cases be used directly, for example in PCR or other amplifications, without eluting the nucleic acid from the support. Also, for many DNA detection or identification methods elution is not necessary since although the DNA may be randomly in contact with the bead surface and bound at a number of points by hydrogen bonding or ionic or other forces, there will generally be sufficient lengths of DNA available for hybridisation to oligonucleotides and for amplification.

However, if desired, elution of the nucleic acid may readily be achieved using known means, for example by heating, eg. to 65°C for 5 to 10 minutes, and

Example 1

DNA isolation from cell culture

$4x10^6$ HL60 cells were washed twice in PBS and pelleted. The pellet was dissolved in 10 µl PBS, and 1 mg of Dynabeads® M-280* obtainable by autoclaving a suspension of Dynabeads® M-280 tosylactivated (available from DYNAL A/S, Oslo, Norway) in water) resuspended in 0.1 ml lysisbuffer [5% SDS/10 mM TrisCl pH 8.0/1 mM EDTA] was added. This was followed immediately by the addition of 1 ml lysisbuffer, and the suspension was incubated for 5 minutes at room temperature, after which the Dynabeads®, with bound DNA was attracted to a magnet and the liquid phase removed. The solid phase was then washed twice with 1 ml washing buffer [50 mM NaCl/10 mM TrisCl pH 8.0/1 mM EDTA]. Finally, the beads, with bound DNA, were resuspended in 0.1 ml water, and incubated for 5 minutes at 65°C. The beads were attracted to a magnet, and the liquid phase withdrawn. The liquid phase was then analyzed for its DNA content. Results from an optical density scan (Fig. 1) are in accordance with pure DNA. The OD_{260}/OD_{280} ratio is 1.72; pure DNA in water or TE has a ratio of 1.7 - 1.9. With pure DNA, the concentration can be determined from the OD_{260} of the solution. A 50 µg/ml solution has OD_{260} = 1.0. From the OD_{260} measurement (Table 1) of 0.436 (0.1 ml total volume, 10 mm lightpath), the yield can be calculated to 2.18 µg DNA, 82% of the 2.67 µg that was the estimated DNA content of the starting material. Gel electrophoresis of a sample of the isolated DNA (Fig. 2) shows that most of it is in a high molecular weight form (>20 kb).

Example 3

Example 1 was repeated using the following combination of lysisbuffers and washing buffers, and the following results were obtained:

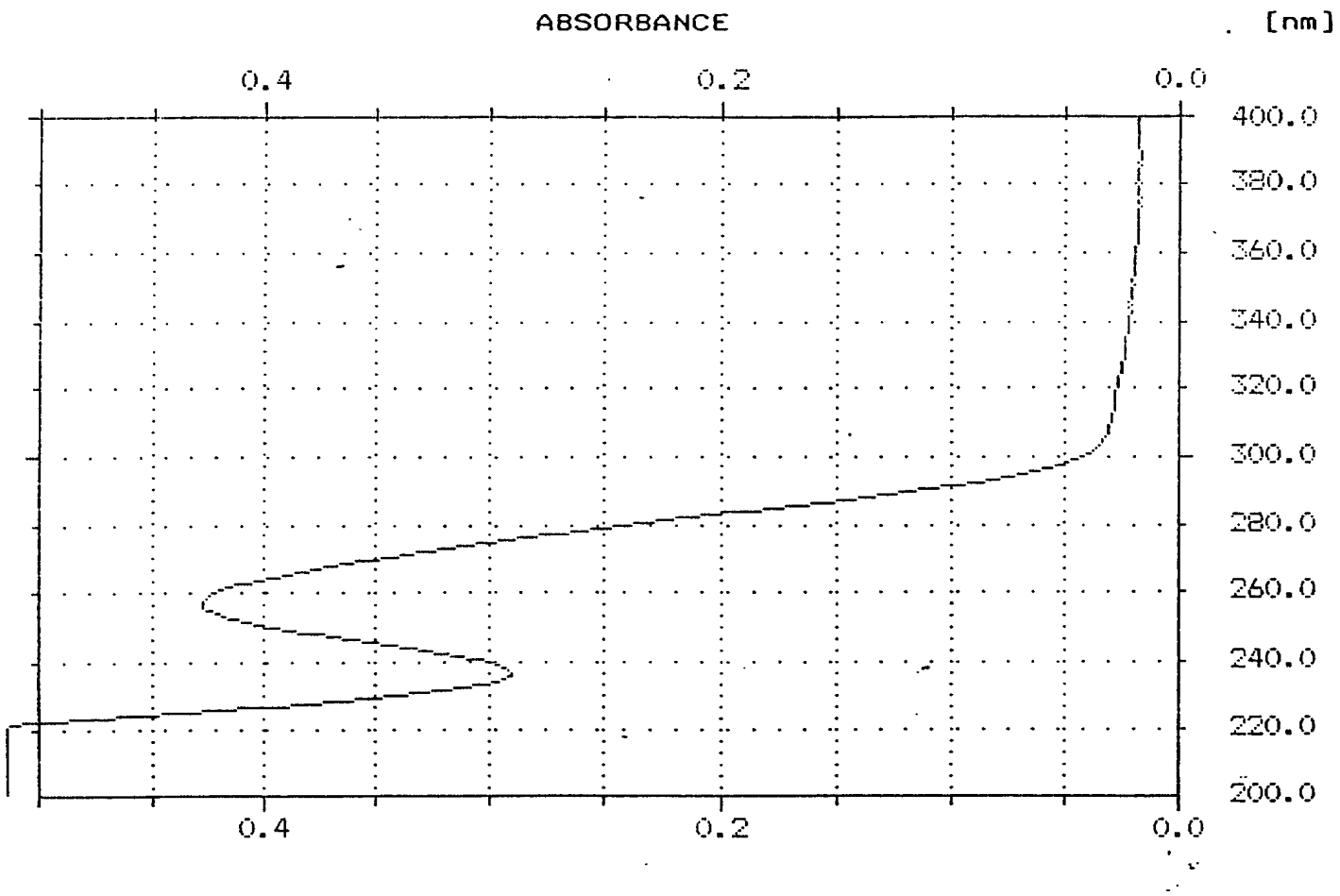
(where +++ indicates very good DNA isolation)

Lysis buffer	Washing buffer	Result
2% SDS	50 mM NaCl/1 x TE	+++
2% SDS/1 x TE	50 mM NaCl/1 x TE	+++
2% SDS/1 x TE/10 mM NaCl	50 mM NaCl/1 x TE	+++
5% SDS	50 mM NaCl/1 x TE	+++
5% SDS/1 x TE	50 mM NaCl/1 x TE	+++
5% SDS/1 x TE/10 mM NaCl	50 mM NaCl/1 x TE	+++
1% LiDS/10 x TE/0.5 M LiCl	50 mM NaCl/1 x TE	+++
1% LiDS/10 x TE/0.5 M LiCl	150 mM LiCl/1 x TE	+++
5% LiDS	150 mM LiCl/1 x TE	+++
5% SDS	150 mM LiCl/1 x TE	+++
1% Sarcosyl	150 mM LiCl/1 x TE	+++

1 x TE is 10 mM TrisCl pH 8.0/1 mM EDTA, 10 x TE is 100 mM TrisCl pH 8.0/10 mM EDTA

Example 4

Following the procedure of Example 1, similar results may be achieved using Dynabeads® M-450 uncoated (Dynal A/S, Oslo, Norway)



THRESHOLD : 0.100

SAMPLE	CYCLE	WAVELENGTH	DATA
004	15:40	257.6 nm (MAX)	0.427 ABS
		236.4 nm (MIN)	0.292 ABS

FIG 1

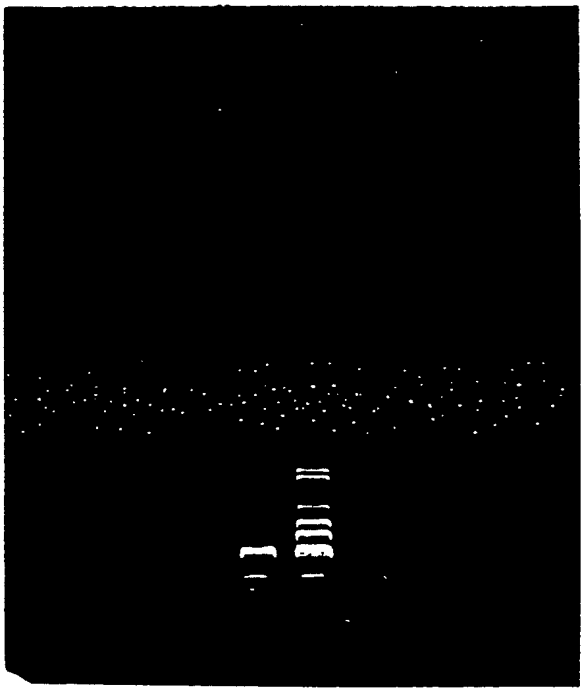


FIG 2

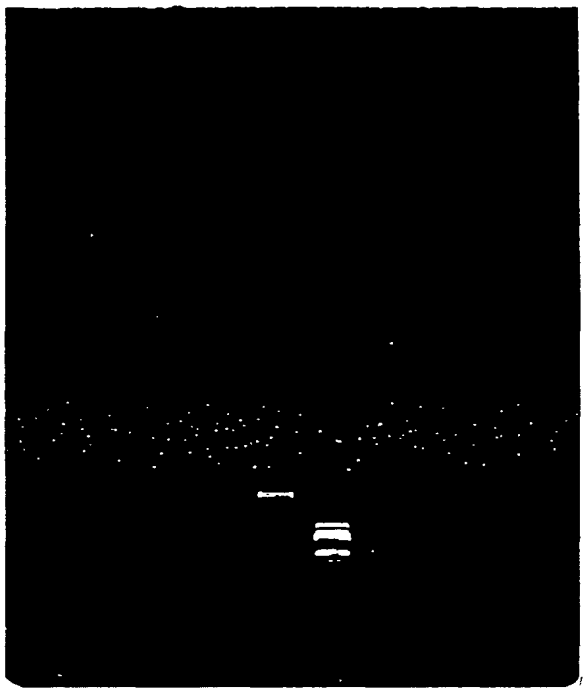


FIG 3

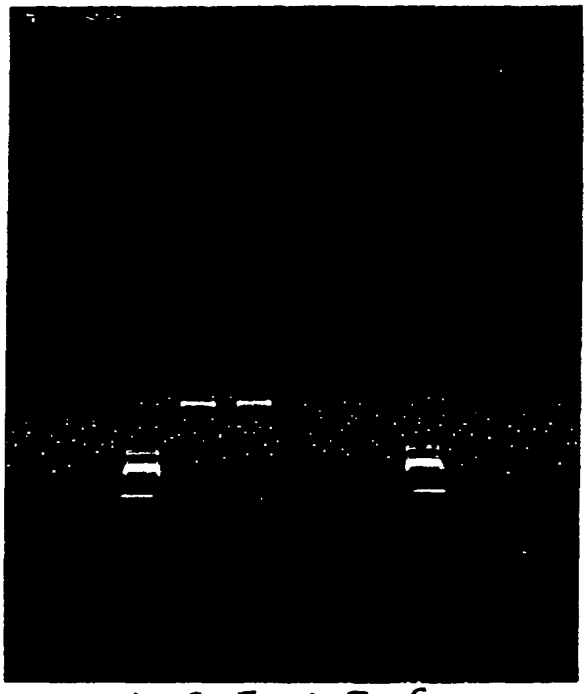


FIG 4

CRUIKSHANK & FAIRWEATHER

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9 December, 1997

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Dear Sirs

International Patent Application Nos. GB96/02937 & GB96/02467
in the name of Award Plc et al

General Power of Attorney - HAMILTON, Ronald Shade

In accordance with written instructions of Ronald Shade Hamilton we hereby renounce in accordance with rule 90.5 (d) (PCT) the General Power of Attorney executed by Ronald Shade HAMILTON dated 26th August 1996 in favour of our firm

Please note this renunciation does not relate to the General Power of Attorney executed by Ronald S Hamilton in his capacity as Chairman and Managing Director of Award Plc dated 26th August 1996.

Please acknowledge that the General Power of Attorney is renounced.

Yours faithfully

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